# Three-MHz Ultrasound Heats Deeper Into the Tissues Than Originally Theorized

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Objective: To measure muscle temperature of ultrasound at 1-MHz and 3-MHz frequencies at a depth of 2.5 cm and to compare treatment durations for vigorous heating (increase of 4°C) and for heating to 40°C.

Design and Setting: A counterbalanced, repeated-measures design with 1 fixed, independent variable, 1.5-W/cm<sup>2</sup> ultrasound treatment (1 MHz, 3 MHz, or control [sham]) using a Theratouch 7.7 ultrasound device. Dependent variables were end-treatment temperature at 2.5 cm, time to vigorous heating, and time to reach 40°C.

**Subjects:** Eighteen healthy volunteers (age =  $24.6 \pm 2.3$ years, height = 173.0  $\pm$  9.7 cm, mass = 72.0  $\pm$  16.3 kg) without a history of lower leg injury.

Measurements: The medial triceps surae intramuscular temperature at 2.5 cm was measured every 10 seconds using an implantable thermocouple. Each of the 3 ultrasound frequencies was applied in counterbalanced order at 24-hour intervals.

Results: Ultrasound of 3 MHz produced both vigorous heating (at 3.4 minutes) and an absolute temperature of 40°C (at 4

Conclusions: Our results suggest that 3-MHz ultrasound heats 0.5 cm deeper than suggested by others. With our machine, 3-MHz ultrasound was more effective in heating muscle at this depth than 1-MHz ultrasound.

Key Words: thermal ultrasound, half-value layer, intramuscular temperature

herapeutic ultrasound has been used extensively to treat a variety of conditions because of its documented thermal effects.<sup>1–7</sup> It has repeatedly been shown to increase tissue temperature at depths up to 5 cm with only minimal increases in skin temperature. <sup>7–11</sup> It has been suggested <sup>10–13</sup> that an increase of 1°C (mild heating) over baseline muscle temperature of 36°C to 37°C accelerates the metabolic rate in tissue. An increase of 2°C to 3°C (moderate heating) reduces muscle spasm, pain, and chronic inflammation and increases blood flow. 10-15 Vigorous heating, or an increase of 4°C or more, has been suggested to alter the viscoelastic properties of collagen and inhibit sympathetic activity. 10-13 Because of baseline temperature differences between individuals, however, it may be better to speak of the thermal effects of therapeutic ultrasound as occurring at specific absolute tissue temperatures rather than relative changes from baseline temperature. 16 For example, many of the authors 3,16-21 who performed the early work on thermal effects described the desired physiologic effects as occurring at an absolute tissue temperature higher than 39.6°C. Regardless of whether we discuss absolute or relative temperature changes, producing a therapeutic increase in tissue temperature requires careful attention to the specific ultrasound settings being used.<sup>7–14</sup>

One of the most important of these settings is ultrasound frequency. Frequency is selected based on the depth of the tissue to be treated. 13,14,22 The depth of ultrasound penetration is usually described in terms of the half-value depth for the specific ultrasound frequency. 13,23 The half-value depth is the distance at which 50% of the ultrasound energy has been dissipated. 13,23 Ultrasound devices have been described as producing therapeutic heating at depths between 1 and 2 halfvalue depths. 13,23 Therefore, 1-MHz continuous ultrasound, with a half-value depth of approximately 2.3 cm, is frequently used to treat deep tissues that are approximately 2.3 to 5 cm deep.<sup>13</sup> With its smaller half-value depth, 3-MHz ultrasound is frequently used to heat tissues that are more superficial, from 0.8 to 1.6 cm deep. 13,23

With 3-MHz treatments typically used for superficial tissues to a depth of about 1.6 cm and 1-MHz treatments typically used for depths greater than roughly 2.5 cm, the appropriate frequency for treating medium-depth tissues is unclear. This is problematic because ultrasound is commonly used to treat tissues, such as the gastrocnemius and wrist extensor group, that are at this depth. Thus, it would be useful to know which ultrasound frequency is the better clinical choice for medium or intermediate treatment depths.

Speculating that 3-MHz ultrasound might penetrate slightly deeper than twice its half-value depth, we chose 2.5 cm to allow us to draw conclusions about intermediate depths, slightly deeper than for 3 MHz and near the shallow margin for 1 MHz. Therefore, our primary purpose was to compare ultrasound frequency with regard to temperature reached and the time needed to produce therapeutic heating at a depth of 2.5 cm. Our secondary purpose was to determine if a difference existed between the treatment duration required to generate vigorous heating (increase of  $4^{\circ}$ C) and the absolute temperature of  $40^{\circ}$ C.

#### **METHODS**

#### Design

We used a  $1 \times 3$  factorial, repeated-measures design. The single independent variable was ultrasound frequency, with 3 fixed levels: 1 MHz, 3 MHz, and control (sham). The treatment order was counterbalanced with a Latin square. The 3 dependent variables were the ending temperature at the conclusion of a 10-minute treatment, the time to increase tissue temperature 4°C from baseline, and the time to reach a temperature of 40°C. All temperature measurements were taken at a depth of 2.5 cm from the ultrasound application surface.

#### Subjects

A group of 18 student volunteers (age =  $24.6 \pm 2.3$  years, height =  $173.0 \pm 9.7$  cm, mass =  $72.0 \pm 16.3$  kg) participated in this study. A brief health-status questionnaire was used to collect subject demographic data and to rule out any excluding factors, including illness, blood-borne disease, and recent history of left leg ecchymosis, infection, edema, or injury. All subjects were informed of the possible risks associated with participation in the study and provided written informed consent for their participation. The School of Health and Human Performance Human Subjects Institutional Review Committee at Indiana State University approved the procedures.

#### Instruments

A recently calibrated Theratouch 7.7 ultrasound device (Rich-Mar, Inola, OK) with a Therapy Hammer transducer capable of producing ultrasound at both 1- and 3-MHz frequencies was used for all treatments. Although the ultrasound unit has 2 transducer head sizes, we only employed the 5-cm² size in this study. The manufacturer reported an effective radiating area of 5 cm,²4 yet we realized that it had to be smaller than the soundhead, so the actual effective radiating area was undetermined. The manufacturer reported a maximum beam non-uniformity ratio of 5.5:1 for this model,²4 but the actual beam nonuniformity ratio for the transducer was not determined.

Intramuscular tissue temperature measurements were taken using implantable Type-T thermocouples (model TX-23–21, Columbus Instruments, Columbus, OH) affixed to a portable Datalogger (MSS-3000, Commtest Instruments, Christchurch, New Zealand). This equipment is reported to be accurate within 1% in the temperature range studied. Thermocouples were disinfected with CidexPlus (Johnson & Johnson Medical, Arlington, TX) between uses. The coupling medium for each treatment was 5 mL of room-temperature UltraPhonic ultrasound transmission gel (Pharmaceutical Innovations, Inc, Newark, NJ).

#### **Procedures**

With aseptic technique and universal precautions, a single thermocouple was inserted into the medial side of the triceps surae horizontally and parallel to the ultrasound treatment surface at a depth of 2.5 cm from the treatment surface using a technique previously described.<sup>26</sup> Horizontal insertion into the side of the medial calf allows the ultrasound to be applied without the sound transducer becoming entangled in the thermocouple lead wire. All thermocouple insertions were performed by the same investigator as follows: A 10-cm-diameter thermocouple insertion area on the left medial triceps surae muscle group was shaved and thoroughly cleaned using 70% isopropyl alcohol. While the subject lay prone, the investigator selected the site for the ultrasound treatment and used a carpenter's square to determine the appropriate location (depth = 2.5 cm from the ultrasound treatment surface) within the shaved area on the medial side of the calf for thermocouple insertion. A single thermocouple was inserted using a sterile 21-gauge by 3.81-cm (1.5-in) hypodermic needle. To ensure that the needle was introduced into the tissue parallel to the treatment surface, a level was used as a guide. After thermocouple insertion, the needle was withdrawn, leaving only the thermocouple in place. The thermocouple was inserted far enough into the calf that it would lie in the center of the ultrasound treatment area. The intratissue location of the thermocouple was verified by measuring the distance from the insertion surface to a mark made on the thermocouple lead.<sup>26</sup>

A template cut to approximately twice the area of the transducer head of the ultrasound applicator was placed onto the skin overlying the treatment area. This ensured that the area for each ultrasound treatment was consistent. After we implanted the thermocouples and connected them to the Datalogger, we recorded baseline tissue temperature for 2 minutes before beginning ultrasound. All baseline and experimental temperature measurements were taken at 10-second intervals.

Ultrasound treatments of 1 MHz, 3 MHz, or control (sham) were administered in a repeated, single-blind fashion at 24hour intervals in an order determined by a Latin square. All treatments were administered with the subject prone, and all used 5 mL of room-temperature ultrasound gel as the coupling agent. Except for the ultrasound frequency, the following identical treatment settings were used: intensity =  $1.5 \text{ W/cm}^2$  (except for the control), continuous duty cycle, and treatment area = twice the size of the transducer head faceplate. For the control treatment, the transducer head was moved over the treatment area, but the ultrasound unit was not turned on, and no acoustic energy was delivered. The ultrasound transducer was moved in a superior-inferior direction within the template at a rate of 3 to 4 cm/s, as previously established.8-10,13,26 Movement speed of the transducer was regulated using a metronome.<sup>26</sup> Instantaneous temperature was recorded every 10 seconds during each application. For subject-safety purposes, the treatments continued until one of the following criteria was met: treatment duration of 10 minutes, the intramuscular temperature remained stable for 6 consecutive samples (1 minute), the subject reported that the treatment was uncomfortable, or an absolute intramuscular tissue temperature of 40°C was reached. Once the ultrasound application was completed, the thermocouple was removed, the insertion area was cleaned using 70% isopropyl alcohol, and an adhesive bandage was applied to the needle-insertion site.

#### **Statistical Analysis**

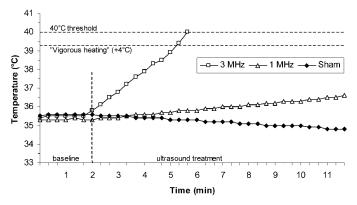
Initially, our multivariate design called for a repeated-measures multiple analysis of variance to identify any differences

### Baseline and End Treatment Temperatures (°C) for Ultrasound Treatments

Treatment*	Baseline	End Treatment†
1 MHz	$35.3\pm0.4$	$36.6\pm0.3$
3 MHz	$35.5 \pm 0.4$	$40.0 \pm 0.0$
Control (sham)	$35.6 \pm 0.4$	$34.8 \pm 0.3$

<sup>\*</sup>Intensity = 1.5 W/cm², effective radiating area =  $5 \text{ cm}^2$ , treatment area =  $2 \times$  effective radiating area, duty cycle = 100%.

<sup>†</sup>Treatment ended at 10 minutes for 1-MHz ultrasound and control and when temperature reached 40°C for 3-MHz ultrasound.



Tissue temperature at 2.5 cm with 1.5-W/cm<sup>2</sup> ultrasound treatments.

in the dependent variables across the treatments. However, the 1-MHz treatment did not heat the tissues to either the vigorous heating level ( $\geq$ 4°C) or to an absolute 40°C in the 10-minute trial period. Because 2 of our dependent variables were thereby negated, our intended statistical analysis was not possible. The level of significance for this test was established at P < .05.

Because of the relative speed of heating with the 3-MHz frequency, all 3-MHz treatments were discontinued before 10 minutes. Therefore, a comparison between 3 MHz and 1 MHz was not performed because each treatment ended at a different treatment duration.

#### **RESULTS**

Using the ultrasound device studied here, continuous 1.5-W/cm² ultrasound treatment for 10 minutes at a frequency of 1 MHz produced neither vigorous heating (increase of 4°C) nor an absolute tissue temperature of 40°C in tissues at a depth of 2.5 cm (Table, Figure). On the other hand, 3-MHz ultrasound produced both vigorous heating (increase of 4°C) and an absolute intramuscular tissue temperature in excess of 40°C at a depth of 2.5 cm. Vigorous heating (increase of 4°C) was achieved at an average of 3.35  $\pm$  1.23 minutes, whereas absolute intramuscular tissue temperature of 40°C was accomplished at an average of 4.13  $\pm$  1.69 minutes with 3-MHz ultrasound. Thus, the rate of heating with a frequency of 3 MHz was 1.19°C/min at a depth of 2.5 cm.

#### DISCUSSION

Our investigation had 2 purposes. Our primary purpose was to determine whether the 1-MHz or 3-MHz ultrasound frequency was more effective at increasing intratissue temperatures at a depth of 2.5 cm. As we know, 2.5 cm falls between

the commonly perceived effective penetration depths for these frequencies. Our secondary purpose was to determine if a difference in treatment durations was required to generate vigorous heating (increase of  $4^{\circ}$ C) and the absolute tissue temperature of  $40^{\circ}$ C.

#### **Comparison Between Ultrasound Machines**

Unfortunately, our 1-MHz heating results were not what we expected. Based on other studies, 13,27 the heating rate of 1-MHz ultrasound should be 3 times slower than that for 3 MHz, because it is absorbed 3 times more slowly. For example, when using the Omnisound 3000 (Accelerated Care Plus, Reno, NV), Draper et al<sup>13</sup> found that 3-MHz ultrasound at 1.5 W/ cm2 heated at a rate of 0.9°C/min and 1-MHz ultrasound heated at a rate of 0.3°C/min. 13,28-30 These findings make perfect sense because the crystal is deforming 3 times faster; thus, the energy should be absorbed 3 times faster. Yet, if we compare our data using the Theratouch 7.7, we see that 3-MHz ultrasound heated at a rate of 1.19°C/min (close to, yet faster than, the Draper et al13 3-MHz data), whereas 1-MHz ultrasound heated at a rate of only 0.13°C/min (much slower than the Draper et al<sup>13</sup> 1-MHz data). This finding is more than 10 times lower and slower than our 3-MHz data. In retrospect, we believe that our ultrasound equipment failed to work optimally during the 1-MHz treatment; therefore, we had to alter our intended analysis regarding which frequency was optimal for heating tissue at 2.5 cm. We understand there is a difference in the quality of ultrasound machines; however, one machine should not produce so much disparity between 2 frequencies.

We did determine, however, that 3-MHz ultrasound penetrated deeper into tissues than originally theorized, and that alone is a new finding. Future researchers will need to determine just how deeply 3-MHz ultrasound heats various tissues (eg, muscle, tendon). It is imperative that we compare machines currently being used in clinical settings, as recommended by Merrick et al.<sup>31</sup>

## 1-MHz Versus 3-MHz Ultrasound at an Intramuscular Depth of 2.5 cm

Draper et al<sup>13</sup> described the thermal effects of ultrasound at both 1 and 2 half-value thicknesses for both 1-MHz and 3-MHz ultrasound. Although Draper et al<sup>13</sup> did not directly suggest that double the half-value layer thickness be used as a limit, this notion is popular and appears to be a guideline for selecting ultrasound frequency based on desired depth of penetration. Stewart<sup>23</sup> suggested that the half-value layer for 3-MHz ultrasound is 0.8 cm deep; thus, it should penetrate up to 1.6 cm deep. Therefore, 3-MHz ultrasound would appear to be reasonably appropriate for treating tissues at depths between 0.8 and 1.6 cm. On the other hand, 1-MHz ultrasound would appear appropriate for treating tissues that are between approximately 2.3 and 5 cm in depth.<sup>13</sup>

However, the use of the half-value layer for determining ultrasound frequency based on tissue depth causes us to raise the question of which frequency should be used for tissues between 1.6 and 2.3 cm deep. In an effort to draw inferences about this entire unknown range, we chose to examine thermal effects at 2.5 cm, a depth at the deepest boundary of the unknown window. At this depth, we observed that 3-MHz ultrasound was adequate in both heating tissues to an absolute temperature of 40°C and increasing the temperature by 4°C from

baseline. In fact, these temperatures were reached rather quickly with this frequency (see Figure). Thus, we conclude that 3-MHz ultrasound is an appropriate choice for depths up to (and perhaps beyond) 2.5 cm, even though these are greater than double the reported half-value thickness.<sup>23</sup>

#### **Ultrasound Depths in Modality Texts**

Many current textbooks are vague about the heating differences associated with ultrasound frequency. Denegar<sup>32</sup> stated that "ultrasound at higher frequencies affects tissues that are more superficial, whereas at a lower frequency less energy is absorbed superficially and more is available to penetrate into deeper tissues." Although this is a fair explanation of the differences in ultrasound frequency, it suggests (with a graph reprinted with permission from Castel) that 1-MHz ultrasound penetrates and heats intramuscular tissues at 1.5 cm and deeper, whereas 3-MHz ultrasound only heats tissues from 0.3 cm to 1.5 cm. Starkey<sup>33</sup> stated that 1-MHz ultrasound can affect tissues up to 5 cm deep, and 3-MHz ultrasound is effective on tissues up to 2 cm deep. Additionally, Draper and Prentice<sup>34</sup> wrote, "at 3 MHz the energy is absorbed in the more superficial tissues, with a depth of between 1 and 2 cm, making it ideal for treating superficial conditions such as plantar fasciitis, patellar tendonitis, and epicondylitis." Thus, 2 cm remains the maximal depth at which temperature changes can occur with 3-MHz ultrasound as represented in each of these texts.<sup>32-34</sup> However, our study revealed that 3-MHz ultrasound effectively heated human tissues at even deeper levels (2.5 cm) than reported in the texts. Therefore, future researchers should address exactly how deeply 3-MHz ultrasound can effectively heat because current texts and literature appear to misrepresent the potential depth of penetration for this ultrasound frequen-

## Relative Temperature Change Versus Absolute Temperature

The secondary purpose of the study was to identify whether a meaningful difference existed between the treatment duration needed to produce vigorous heating (4°C relative change) and the duration needed to reach 40°C absolute temperature. Although we wanted to examine this issue for both common ultrasound frequencies, we were only able to compare with 3-MHz ultrasound because our 1-MHz ultrasound treatment did not reach either target.

For our 3-MHz treatment, subjects started with an average baseline temperature of 35.5°C, and we observed a significant difference of approximately 39 seconds between reaching vigorous heating (3 minutes, 21 seconds into the treatment) and reaching 40°C (4 minutes). These results amounted to a 16% difference in treatment duration. Although the difference was statistically significant, we must examine the meaningfulness of the difference. That is, does a 39-second difference meaningfully affect the outcome of a 3-MHz ultrasound treatment? We suggest that it probably would not have affected the outcome in this case because vigorous heating was reached at 3.4 minutes, whereas 40°C was reached at 4 minutes. Typically, 3-MHz ultrasound treatments last 5 minutes at the intensity we used (1.5 W/cm<sup>2</sup>), so both the relative change target (reached at 3.35 minutes) and the absolute temperature target (reached at 4 minutes) would have been achieved. Therefore, it makes little difference clinically whether the target is relative or absolute with a 3-MHz ultrasound at the intermediate depth of 2.5 cm, provided the protocol is designed to overshoot the target enough to allow for a reasonable margin of error.

#### **CONCLUSIONS**

Our primary conclusion is that 3-MHz ultrasound penetrates tissues more deeply than originally theorized. More research with various ultrasound machines needs to be performed to see if 3-MHz ultrasound is more appropriate than 1-MHz ultrasound for producing thermal effects in tissues 2.5 cm deep. Researchers can also explore the maximum depth to which 3-MHz ultrasound can adequately heat various tissues.

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